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Clustering of Cases of Insulin Dependent Diabetes (IDDM) Occurring Three Years After Hemophilus Influenza B (HiB) Immunization Support Causal Relationship Between Immunization and IDDM

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Objective: The hemophilus vaccine has been linked to the development of autoimmune type 1 diabetes, insulin dependent diabetes (IDDM) in ecological studies.

Methods: We attempted to determine if the Hemophilus influenza B (HiB) vaccine was associated with an increased risk of IDDM by looking for clusters of cases of IDDM using data from a large clinical trial. All children born in Finland between October 1st, 1985 and August 31st, 1987, approximately 116,000 were randomized to receive 4 doses of the HiB vaccine (PPR-D, Connaught) starting at 3 months of life or one dose starting after 24 months of life. A control–cohort included all 128,500 children born in Finland in the 24 months prior to the HiB vaccine study. Non-obese diabetic prone (NOD) mice were immunized with a hemophilus vaccine to determine if immunization increased the risk of IDDM.

Results: The difference in cumulative incidence between those receiving 4 doses and those receiving 0 doses is 54 cases of IDDM/100,000 ($P = 0.026$) at 7 years, (relative risk = 1.26). Most of the extra cases of IDDM appeared in statistically significant clusters that occurred in periods starting approximately 38 months after immunization and lasting approximately 6–8 months. Immunization with pediatric vaccines increased the risk of insulin diabetes in NOD mice.

Conclusion: Exposure to HiB immunization is associated with an increased risk of IDDM. NOD mice can be used as an animal model of vaccine induced diabetes.

Keywords: Insulin dependent diabetes; Vaccines; Immunization; Hemophilus

INTRODUCTION

We discovered a rise of type 1, insulin dependent diabetes (IDDM) occurred in Finland following the introduction of the Hemophilus influenza B (HiB) vaccine.[1] Due to the low relative risk associated with a single vaccine, we wanted to determine if we could identify specific clusters of cases of IDDM associated with the hemophilus vaccine. We initiated toxicity studies in non-obese diabetic prone (NOD) mice to determine if the vaccine could increase the risk of diabetes in the mice and if the findings in mice correlated with the findings in humans.

METHODS

We followed upon a clinical trial described in detail earlier.[2] All children born in Finland between October 1st, 1985 and August 31st, 1987, approximately 116,000, were randomized to receive 4 doses of the HiB vaccine (PPR-D, Connaught) starting at 3 months of life (3, 4, 6, 18 months) or 1 dose starting at 24 months of life. In the latter group, the mean age of immunization was approximately 26 months of life. By design of the original study, historical controls were designated as the unvaccinated controls for long-term safety studies.

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TABLE 1 Incidence of IDDM in children receiving 4, 1, or 0 doses of hemophilus vaccine

	4 Doses (59,024)		1 Dose (56,921)		4 or 1 Dose (115,945)		0 Dose (128,532)							
Population	Cases IDDM	Cumulative incidence (100,000)	Cases IDDM	Cumulative incidence (100,000)	Cases IDDM	Cumulative incidence (100,000)	Cases IDDM	Cumulative incidence (100,000)	Incidence difference (4 doses vs. 0 dose)	P* value (4 vs. 0)	Incidence difference (4/1 doses vs. 0 dose)	P* value (4 or 1 vs. 0)	Incidence difference (1 doses vs. 0 dose)	P* value (1 vs. 0)
Age														
0–7	154	261	135	237	289	249	266	207	54	0.024	42	0.030		
0–5	98	166	83	146	181	156	180	140	26		16			
0–2	21	36	21	37	42	36	33	26	10		11			
5–10	137	232	131	230	268	231	257	200	32		31	(0.053)		
2–7	133	225	114	200	247	213	253	181	44	(0.026)	32	(0.042)		
2–5	77	130	62	109	139	120	147	114	16		6			
5–7	56	95	52	91	108	93	86	67	28	(0.0269)	26	0.026	24	(0.048)
7–10	81	137	79	139	160	138	171	133	4		5			
0–10	235	398	214	376	449	387	437	340	58	(0.029)	47	(0.028)		

*Statistics: two tailed or (one tailed) Fisher calculated using WHO/CDC's EPI-6 program.

The control group, which did not receive the HiB vaccine, included all 128,500 children born in Finland in the 24 months prior to the HiB vaccine study. We used an intent to treat method to calculate the incidence of IDDM in each group until age 10. The initial design of the study called for determining the cumulative incidence of IDDM up to an age of 7 years, however, additional information became available and we were able to follow children for the incidence of IDDM up to 10 years of age. Graphical data was analyzed for the presence of clusters. Cases of IDDM were collected as part of a prospective registry described in detail earlier,[3,4] and data on the yearly incidence of IDDM from this registry was analyzed for patterns predicted by the clinical trial data.

Animal Study

Non-obese diabetic (NOD) mice spontaneously develop an autoimmune destruction of their pancreatic islet cells leading to IDDM, and are considered a good model of human disease. Pregnant NOD/MrkTactBR mice were purchased from Taconic (Germantown, NY) and their offspring received the immunization schedules described below. Animals were housed in pathogen free environment. The following vaccines were used: combined diphtheria, tetanus and acellular pertussis vaccine DTaP (SmithKline Beecham); hepatitis B (SmithKline Beecham), inactivated polio (Pasteur Merieux), HiB, ActHIB (HiB) (Pasteur Merieux). All female NOD mice received hepatitis B vaccine diluted in buffered saline (1:20) and were injected with 0.1 ml intraperitonealy on day 3 of life and intramuscularly on day 28 of life. The treatment (vaccinated) group ($n = 40$) received additional doses of the DTaP & hemophilus & inactivated polio vaccine which had been mixed 1:1:1 and then diluted 1:50 and given 0.2 ml intramuscularly on weeks 10, 16, 22. Mice in the control group ($n = 37$) were injected with 0.2 ml of saline intramuscularly on weeks 10, 16, 22.

Mice were followed for the development of diabetes from 10 weeks of age through week 32 of age. The urine of mice was tested for glucose on a weekly basis using a glucose sensitive stick (Bayer Diastick). Animals with a reading of 3 or greater on two consecutive weeks were considered diabetic. Mice diagnosed with diabetes by urine were confirmed to be diabetic by testing blood from a tail bleed using a glucose sensitive stick (Lifescan, One Touch, Johnson and Johnson).

Statistics

The relative risks and p values in the epidemiology study were calculated using Epi 6 software (WHO) and the Fisher and chi square tests. Survival analysis was performed on animal data using Statisitica software and the Wilcoxon test.

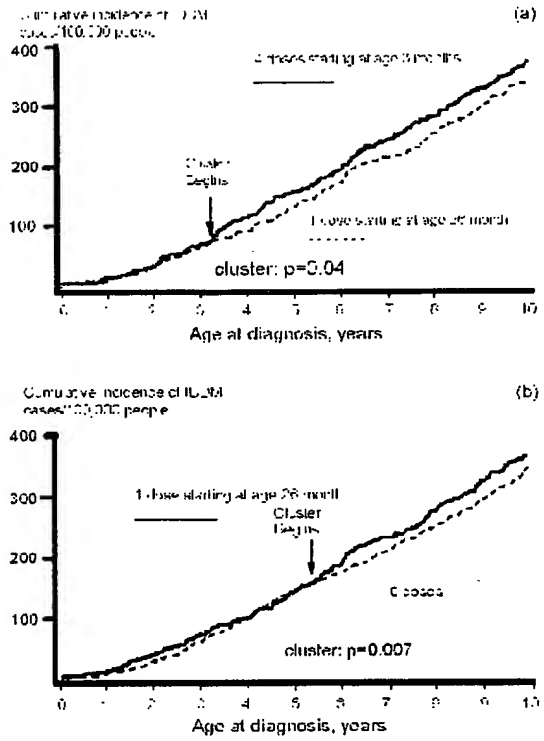

FIGURE 1 (a) Children received 4 doses (3, 4, 6, 18 months) or 1 dose (26 months) of the HiB vaccine and were followed from birth through age 9 of life (10th birth day) for the development of IDDM. (b) Children received 1 dose (26 months) or 0 doses of the HiB vaccine. Children were followed from birth through age 9 of life (10th birth day).

RESULTS

Human Data

The results of the study are described in detail in Table I, Figs. 1a, b and 2. The cumulative incidence of IDDM/100,000 in the groups receiving 4, 1, and 0 doses of hemophilus vaccine are 261, 237, 207, respectively, at 7 years, a prospectively defined end point. The difference in cumulative incidence between those receiving 4 doses and those receiving 0 doses is 54 cases of IDDM/100,000 ($p = 0.024$, two tail test) at 7 years. The relative risk equals 1.26 (1.03–1.54) at 7 years. The difference in cumulative incidence between those receiving any vaccine (4 or 1 doses) and those receiving 0 doses is 42 cases IDDM/100,000 ($p = 0.030$, two tail test) at 7 years, relative risk = 1.2 (1.02–1.42).

Additional follow up showed the curves diverged only slightly more between ages 7 and 10. However, the power of the study declined because of an increase in the underlying number of cases of diabetes. The differences between groups at 10 years were only statistically significant using a single tailed test. The cumulative incidence of IDDM/100,000 in the groups receiving 4, 1, and 0 doses of hemophilus vaccine are 398, 376, 340 at 10 years of age, respectively. The difference in cumulative incidence between those receiving 4 doses and those receiving 0 doses is 58 cases IDDM/100,000 at 10 years ($P = 0.029$ single tail, 0.058 two tailed). The relative risk is 1.17 at 10 years. The difference in cumulative incidence between those receiving any vaccine (4 or 1 doses) and those receiving 0 doses is 47 cases of IDDM/100,000 ($p = 0.028$ single tail, $p = 0.056$ two tailed) at 10 years, relative risk = 1.14.

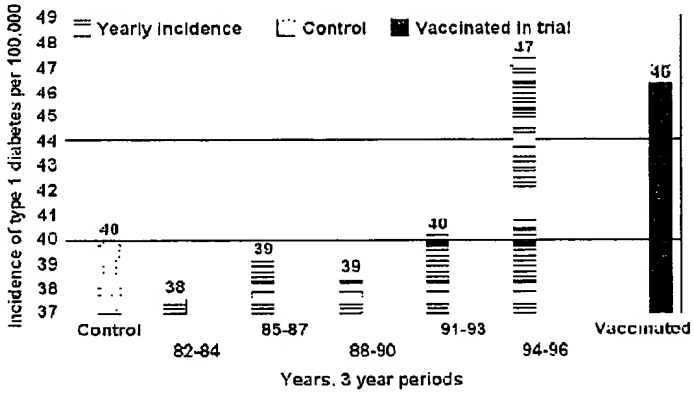

FIGURE 2 The yearly incidence of IDDM in Finland in children age 5 through age 9 of life from 1982 to 1996 is tabulated in 3 year averages. The incidence of IDDM in the unvaccinated and vaccinated group in the clinical trial is compared to the underlying incidence of IDDM in Finland. The incidence in the control group is comparable to the underlying incidence of IDDM in Finland prior to HiB vaccinated children reaching age 5. The incidence of IDDM in Finland rose after the vaccinated children reached age 5 (1994–1996) and the incidence in Finland equaled that in the vaccinated group from the clinical trial.

Graphical data (Fig. 1a, b) shows the curves initially have little or no separation, separate at specific reflection points, then become almost parallel. Data in Table I shows that there is a difference in the cumulative incidence of IDDM of 22 cases/100,000 by age 10 between groups receiving 4 and 1 dose of HiB vaccine. This difference had been stable since before age 5. Table I shows that all the differences between the cumulative incidence of IDDM between the two groups occurs between ages 2 and 5. Furthermore, Fig. 1a shows that the curves separate at approximately 39 month of age then become parallel. Essentially all the difference between the cumulative incidence curves of the 4 and 1 dose curves is clustered and occurs during an 6 month period which starts at 39 month of age. Analysis of this cluster reveals the curves separate by approximately 20 cases/100,000 during this a span lasting about 6 months ($p = 0.04$), relative risk 2.25 ($1.03 < RR < 4.91$).

An similar and statistically significant cluster also occurs in the group receiving 1 dose of vaccine at 26 months of life. Figure 1b shows the cumulative incidence of IDDM curves for the group receiving 1 dose of HiB vaccine and the control group receiving 0 doses. The curves had minor separation prior to 5 years of age, however the curves became superimposable between ages 5 and 5.5 years. Around 5.5 years of age (approximately 40 months after immunization) a cluster of extra cases of IDDM occurred in the group receiving 1 dose of HiB vaccine. Table I shows that there is a cluster occurring between age 5 and 7 where the incidence of IDDM in the group receiving 1 dose increases over the unvaccinated control by 24 cases/100,000. By contrast, the curves only separate by 6 cases between ages 7 and 10. The analysis of the cluster indicates that the curves separate by approximately 24 cases/100,000 over a span lasting about 7.6 months and it is statistically significant ($p = 0.007$), relative risk of 2.17 ($1.27 < RR < 3.73$).

Ecological data on the incidence of IDDM from Finland are consistent with a rise in the incidence of IDDM following hemophilus immunization. The annual incidence of IDDM in the age group 5 through 9 had been stable[3,4] at approximately 39 cases/100,000/year from 1983 to 1993 (Fig. 2). This incidence is almost identical with what was found in the unvaccinated control group, an average incidence of 40 cases/100,000/year. In contrast, the HiB vaccinated groups (both the 1 and 4 dose groups) had an average incidence of 46 cases/100,000/year over these 5 years. Further follow-up of the ecological data shows the incidence of IDDM in the 5 through 9 age group stabilized at approximately 47 cases/100,000 (range 46.5–48.3) between the years 1994 and 1996.[4]

Animal Data

Two mice in the treatment group died before developing diabetes at week 11 and 22, respectively, all other mice were followed through week 32 of age for the development of diabetes. The group of mice receiving the hemophilus vaccine in conjunction with the DTP vaccine and inactivated polio developed diabetes at a higher rate ($p = 0.02$) compared to the control group (Fig. 3).

DISCUSSION

>The data shows a statistically significant association between the hemophilus vaccine and an increased risk of IDDM at a prospective endpoint, 7 years. Most of the extra cases of diabetes associated with immunization appeared in clusters occurring in an period starting approximately 38 (±2) months after the vaccine is given and lasting approximately 6 months. An preexisting underlying temporal rise in the incidence of IDDM in Finland cannot

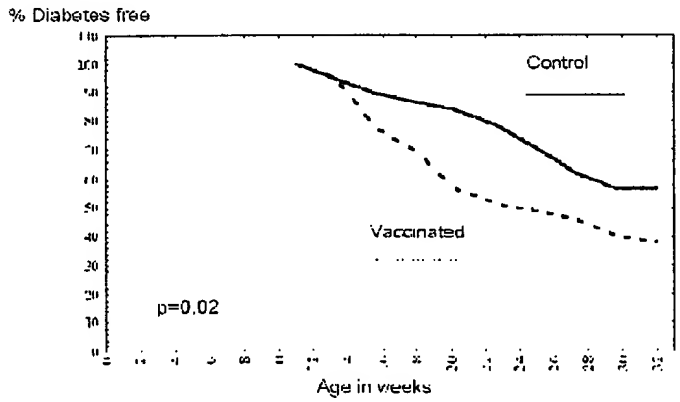

FIGURE 3 All female NOD mice were injected intraperitonealy with hepatitis B vaccine injected on day 3 of life and intramuscularly on day 28 of life. The "vaccinated" group ($n = 40$) received additional doses of the DTaP, hemophilus, and inactivated polio vaccines on weeks 10, 16, 22. Mice in the "control" group ($n = 37$) were injected with saline intramuscularly on weeks 10, 16, 22. Mice were followed for the development of IDDM from week 10 to week 32.

explain the clusters nor can it explain the increased incidence in the 5–9 year old population because the incidence of IDDM had been stable in this age group in Finland prior to the introduction of the vaccine. Animal toxicity data provides additional support for an causal relationship between immunization and IDDM. Our analysis differ from a preliminary analysis of this data[5] which did not fully analyze all sub groups, did not contain cluster analysis and the calculations were actually incorrect.[6] The current findings indicate that further studies are necessary to ensure safe immunization with the hemophilus vaccine.

We had previously reported an association between hemophilus immunization and the development of diabetes in Finland. We performed a cluster analysis after noting clusters in the graphical data. Statistically significant clustering of cases of IDDM occurred in periods starting approximately 38 (±2) months after immunization in both HiB immunized groups. The clusters were similar in both groups with the majority of the extra cases of IDDM associated with immunization occurring in a period lasting about 6–8 months. The graphs (Fig. 1a, b) show the curves are superimposable prior to the cluster and then become almost parallel or diverge slowly after the cluster. There is some random variation in the curves and the actual size and statistical significance of the clusters varied slightly depending on the designated start of the cluster and the duration of the cluster. However, the clusters are clearly evident in the graphs and are statistically significant.

Both the groups receiving 1 and 4 doses of hemophilus vaccine received a dose of vaccine after 18 months of life. The major difference between the groups was that the group receiving 4 doses also received doses at 3, 4, 6 months of life. The cumulative incidence of IDDM curves of the 1 and 4 doses separated at about 39 months of age. A cluster of cases of IDDM occurred in the 4 dose group during the following 6 months which accounts for about an extra 20 cases/100,000 compared to the 1 dose group. Since the groups receiving 1 and 4 doses of HiB vaccine differed by an cumulative incidence of 22 cases of IDDM/100,000 by age 10, all the extra cases associated with the 4 dose group occurred in this cluster. The groups receiving 1 and 0 doses of the HiB vaccine differed by an cumulative incidence of IDDM of 36 cases/100,000 by age 10. Of these, approximately 24 cases/100,000 were clustered in a period of time which started approximately 40 months after the HiB vaccine was given and lasted about 7.6 months.

Ecological data was analyzed to determine if the incidence of IDDM had risen following immunization with the hemophilus vaccine in a manner predictable based on the clinical trial data. If the differences between the groups had been just due to random variation then one would not expect to see a rise in the underlying incidence of IDDM in Finland. We found that while there had been some previous rises in the incidence of IDDM in the 0–4 year olds prior to the introduction of the HiB vaccine, the incidence of IDDM had been stable in the 5–9 age group from 1982 to 1993. As the hemophilus vaccinated group reached age 5, the incidence of IDDM rose sharply in the 5–9 age group and equaled that in the vaccinated group in the clinical trial. This ecological data provides support that the differences between the groups is real and cannot be explained by either an preexisting rise in the incidence of diabetes nor random variation in the groups.

The incidence of IDDM had been rising in Finland prior to the introduction of the hemophilus vaccine and it had been hypothesized that this trend could explain the separation between the groups.[5] The data indicates little if any of the difference between the groups is due to an preexisting underlying rise in the incidence of IDDM. The data indicates the incidence of IDDM in the 5–9 age group had been stable prior to the study as discussed above. While there was a rise in the incidence of IDDM in the 0–4 age group prior to the study, this again cannot explain the differences between the groups. The group receiving 1 dose of vaccine at 26 month would not be expected to differ from the group receiving 0 doses prior to 5 years of age if we assume a 38-month delay between vaccination and onset of IDDM. In fact the groups receiving 1 dose and 0 dose (the historical control) only differed by an cumulative incidence of 6 cases of IDDM/100,000 at 5 years of age, 146 vs. 140 cases/100,000 (Table I). This is the total maximum effect that may be readily explained by an underlying temporal rise in the incidence of IDDM. However, since the curves of the 0 and 1 dose groups became superimposable between age 5 and 5.5 (Fig. 1b) the difference of 6 cases/100,000 is probably related to random variability and not due to an underlying rise in the incidence of IDDM. In addition, a preexisting underlying rise in the incidence of IDDM would not explain why all the extra cases occurred in clusters. Therefore little if any difference between the groups can be explained by an underlying rise in the incidence of IDDM in Finland.

NOD mice receiving the hemophilus vaccine administered in conjunction with the DTaP and inactivated polio vaccines had a higher rate of diabetes than controls. The hemophilus vaccine was not given alone to the mice since it is generally given in combination with other vaccines in humans and we wanted to see the combined effect as we were studying in humans. It is especially important to note that fairly low doses of the vaccines were given to mice. The data support a causal relationship between pediatric vaccines and IDDM. The data also indicates the NOD mice provide a good model for studying the effects of vaccines on IDDM in humans.

Several authors have published studies claiming no association between the hemophilus vaccine and IDDM.[5,7–9] Our analysis indicates a delay between hemophilus vaccination and the development of IDDM of at least 3 years, with an relative risk of around 1.2 with 10 years of follow up. These parameters require that large studies be performed with long-term follow up. The negative studies were severely underpowered, however,

they discover similar relative risks as we detected. The authors concluded that there is not association between the vaccine and IDDM because they did not reach statistical significance and the relative risk is low. We interpret these studies as simply being underpowered, but because the relative risk is similar to our findings, the data actually supports our findings. We make this conclusion because data from several small studies showing the same finding can be pooled and reach statistical significance even when the individual studies do not reach statistical significance on their own.

We believe data from three case control studies, which all reported no association between the HiB vaccine and IDDM, actually support our findings. A multicenter collaborative study looked for an association between the hemophilus vaccine and the development of IDDM.[8] The study involved 900 diabetic children and 2302 controls. The authors found that the hemophilus vaccine was associated with an odds ratio of 1.16.[8] A CDC case control study[9] found that 247 of 260 diabetics received the hemophilus vaccine compared to 733 of 780 controls, odds ratio of 1.22. These results are almost identical to the relative risk of 1.19 at age 5, 1.26 at age 7, and 1.17 at age 10 for the children receiving 4 doses of hemophilus vaccine in Finland. Another case control study looked at the association between development of anti-islet cell auto-antibodies and vaccines.[7] The study was very limited by its design[10] and only studied 25 individuals with an a single auto-antibody and 292 controls. However, even with all these limitations the data showed the hemophilus vaccine associated with an odds ratio of 1.64. All three of these case controls studies were too small to reach statistical significance.

Data from the UK provide further support for an association between the HiB vaccine and IDDM. Just as in Finland there was a rise in the incidence of IDDM following the introduction of the HiB vaccine. The data from the UK shows sharp rises in the incidence of IDDM about 3–4 years following the introduction of the HiB vaccine. The HiB vaccine (PRP-T) was offered to infants in the Oxford regions of the UK starting May 1, 1991 in three of the region's eight districts and July 1, 1991, in a fourth district. Over 90% of infants had been immunized by October 1, 1992.[11] Starting in October of 1992 the vaccine was offered to all children under 5 in the UK.[12] The incidence of IDDM rose 33% acutely in the Oxford region in children under age 5 starting in 1994[13] and continued through 1995. This follows the same approximate 3 year delay between immunization with the hemophilus vaccine in Finland and the rise in IDDM.

There are several mechanisms by which the hemophilus vaccine would be expected to cause type 1 diabetes.[14] One mechanism may be the activation of macrophages which destroy islet cells. Data supporting a causal relationship between macrophage activation and IDDM includes data showing humans at risk for IDDM, because of family history, have increased macrophage activity similar to that seen in diabetics.[15,16] Animal models indicates the macrophages are involved in the initiation of diabetes.[17] Many vaccines activate macrophages and would be expected to alter the risk of IDDM. Vaccines can both directly activate macrophages and indirectly activate macrophages through the release of cytokines. Macrophages are particularly stimulated by vaccine adjuvants including aluminum[18] and complex polysaccharides[19] similar to what are found in certain capsular vaccines like pneumococcal and hemophilus vaccines. Insoluble polysaccharides[19] like those found in vaccines are also more potent activators of macrophages then soluble polysaccharides which may be more common with natural infections.

The data presented here, in conjunction with the related information provide evidence for a causal relationship between the hemophilus vaccine and the development of IDDM. The magnitude of effect is particularly concerning. The PRP-D based HiB vaccine is associated with an extra 58 cases of IDDM/100,000 and the more potent PRP-T HiB vaccine is associated with an even large rise, possibly 75 cases/100,000 by age 10. By contrast hemophilus immunization was initiated in Finland to prevent seven deaths and 7–26 cases of severe disability per 100,000 immunized.[20] Since the long term consequence of IDDM are very morbid, the health of four children may be adversely affected for every child that is benefited when just considering IDDM, and IDDM is just one of many autoimmune diseases that may be influenced by immunization.

In countries lacking modern medical treatment the benefits of the HiB vaccine may exceed the potential risks. However, in industrialized nations changes in HiB immunization should be considered. One possibility is to administer just a single dose. Another possibility is to start HiB immunization in the first month of life.[21] We observed that immunization starting in the first month of life is associated with a decreased risk of IDDM compared to immunization starting after 2 months in both animals and humans[1] and may optimize the trade off between preventing infection and inducing IDDM.

UPDATE

A recently published paper by the US CDC (Pediatrics 108 (6): e112, 2001) confirms their preliminary data that HiB immunization is associated with a odds ratio of approximately 1.17 unadjusted (1.14 and 1.23 adjusted) for possible confounders with respect to IDDM. We have also found two publications (Diabetes Research 9, 111-116 (1988); Journal of Pediatrics 86,654-656, (1975)) describing epidemics of mumps infections followed 3 years later by epidemics of diabetes. These findings are consistent with and provide support for our findings of a 36 month delay between vaccination and the development of diabetes.

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